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Thermo- and pH-Sensitive Aqueous Micellar Gels form a Tertiary Amine-Containing Hydrophilic ABA Triblock Copolymer

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Thermo- and pH-Sensitive Aqueous Micellar Gels from a Tertiary Amine-Containing Hydrophilic ABA Triblock Copolymer

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Abstract

This thesis work reports the synthesis of a well-defined hydrophilic ABA triblock copolymer composed of a poly(ethylene oxide) (PEO) middle block and tertiary amine-containing thermo- and pH-sensitive outer blocks and the study of sol-gel transitions of its aqueous solutions at various pH values. The doubly responsive linear triblock copolymer, poly(methoxydi(ethylene glycol) methacrylate-*co*-ethoxydi(ethylene glycol) methacrylate-*co*-*N,N*-diethylaminoethyl methacrylate)-*b*-PEO-*b*-poly(methoxydi(ethylene glycol) methacrylate-*co*-ethoxydi(ethylene glycol) methacrylate-*co*-*N,N*-diethylaminoethyl methacrylate) (P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)), was prepared by atom transfer radical polymerization of a mixture of DEGMMA, DEGEMA and DEAEMA with a molar ratio of 6:4:1, respectively, from a difunctional PEO macroinitiator. The incorporation of a small amount of tertiary amine-containing monomer into the outer blocks allows their lower critical solution temperature (LCST) to be tuned through pH adjustment. To study the sol-gel transitions, a 10.0 wt % aqueous solution of the triblock copolymer with a pH of 9.65 was made; its pH value was readily adjusted by the injection of a 1.0 M HCl solution. From rheological measurements, the dependence of sol-gel transition temperature ($T_{\text{sol-gel}}$) on pH was found to follow the expected trend. No sol-gel transition was observed at pH 8.6.

Introduction

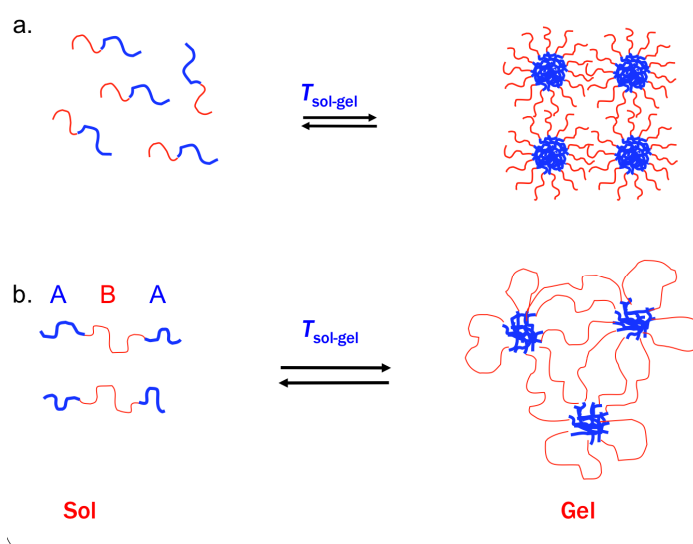
Stimuli-sensitive AB diblock and ABA triblock copolymers have been shown to self-assemble in dilute aqueous solutions into micelles, with the stimuli-responsive A block(s) being associated into the core and the hydrophilic B block forming a corona upon application of stimuli.^{1–10} At a sufficiently high concentration, this sensitivity often manifests as an *in situ* transition from a free-flowing solution to a free-standing gel upon the application of an external trigger. Although both AB diblock and ABA triblock copolymers can be used to make these micellar gels, their gelation mechanisms are different (Scheme 1). For diblock copolymers, the sol-to-gel transition occurs when the volume fraction of micelles in solution exceeds a critical value, and individual micelles are packed into an ordered structure, typically requiring a minimum polymer concentration of around 20 wt %. ABA triblock copolymers, on the other hand, have been shown to form 3-dimensional micellar networks by virtue of the B blocks forming bridges among micellar cores, making the required polymer concentration much lower with respect to analogous diblock copolymers, with critical gelation concentrations as low as 7.5 wt % having been reported.^{1–3,11}

Such stimuli-responsive aqueous micellar gels, particularly those from ABA linear triblock copolymers, have received growing interest in recent years, finding applications in controlled release of drugs, tissue engineering, and others.^{5,6,12–15} This interest can largely be ascribed to the fact that, compared with chemically crosslinked hydrogels, the *in situ* sol–gel transition of these micellar gels coupled with the nature of physical crosslinking allow for more convenient delivery and removal of polymers. Many reports have been made regarding micellar gels of stimuli-sensitive hydrophilic ABA triblock and other block copolymers. Using atom transfer radical polymerization (ATRP), Armes *et al.* synthesized a series of ABA triblock copolymers,

composed of a biocompatible phosphorylcholine-containing polymer as the central block and pH- or temperature-sensitive polymers as outer blocks, capable of forming gels in water in response to either pH or temperature changes.^{16–20} Kirkland *et al.* prepared poly(*N*-isopropylacrylamide)-*b*-poly(*N,N*-dimethylacrylamide)-*b*-poly(*N*-isopropylacrylamide) (PNIPAm-*b*-PDMA-*b*-PNIPAm) by reversible addition fragmentation chain transfer (RAFT) polymerization; above the lower critical solution temperature (LCST) of PNIPAm, aqueous solutions of this triblock copolymer underwent sol-gel transitions resulting in micellar gels with mechanical properties similar to collagen, a naturally occurring polypeptide that has been used as a cell growth scaffold.¹¹

Scheme 1. Gelation Mechanisms of AB Diblock Copolymers (a) and ABA Triblock Copolymers

(b)



Of marked interest to us are aqueous block copolymer micellar gels that can respond to multiple stimuli, as sol-gel transitions capable of being induced by at least two distinct physical or chemical stimuli provide a greater design flexibility needed in many technological and

biomedical applications.¹⁸⁻²⁰ Li *et al.* reported the synthesis of thermo- and redox-sensitive ABA triblock copolymers by ATRP using a difunctional initiator containing a redox-sensitive disulfide bond. Temperature- and pH-sensitive block copolymer aqueous gels are probably the most studied multi-responsive micellar gels, with block copolymers usually prepared by either growing pH-sensitive blocks from or introducing pH-responsive groups to the chain ends of an ABA triblock copolymer that can form thermoreversible gels in water (e.g., PEO-*b*-PPO-*b*-PEO).²¹⁻²⁹ Suh *et al.* used pyromellitic dianhydride to couple PEO-*b*-PPO-*b*-PEO to make multiblock copolymers with carboxylic acid groups being incorporated at the junction points.²⁶ More recently, Lee *et al.* reported pH- and thermo-sensitive aqueous gels of multiblock copolymers composed of PEO and poly(amino urethane).²⁹⁻³¹

Through the incorporation of stimuli-sensitive moieties into the thermo-sensitive outer blocks, which allows the LCST to be tuned via a second stimulus, our lab has recently reported doubly responsive copolymers capable of undergoing multiple sol-gel-sol transitions.^{32,33} It has been shown that the LCST transition temperature of a thermosensitive polymer containing a small amount of weak acid or base groups can be precisely and reversibly tuned by adjusting the solution pH, allowing one to reversibly tune the sol-gel transition temperature in a wide temperature range. Our lab has shown that the incorporation of carboxylic acid groups allowed for the synthesis of ABA block copolymers with a $T_{\text{sol-gel}}$ that increased with pH.³⁴

The goal of this thesis work is the development of thermo- and pH-sensitive, injectable ABA triblock copolymer micellar gels whose sol-gel transition temperature decreases with pH. It is known that the diseased tissues often exhibit unusually acidic pH values.³⁵ Thus, to use injectable hydrogels as drug delivery systems to battle certain diseases, it would be advantageous to employ a weak base with a suitable pK_a for the thermo- and pH-sensitive ABA triblock

copolymers, allowing drugs loaded into the gels to be released at a greater rate at the desired areas (Figure 1). Therefore, the approach taken in this work involves the incorporation of tertiary amine groups into the thermosensitive blocks of an ABA triblock copolymer (Scheme 2). To form a gel at room temperature, the thermosensitive polymer making up the A blocks must have a cloud point of $\sim 10\text{ }^{\circ}\text{C}$, as typical $T_{\text{sol-gel}}$ values of ABA triblock copolymers of this sort have been shown in our prior work to be approximately 10°C above the LCST of the A blocks. Copolymerization of methoxydi(ethylene glycol) methacrylate (DEGMMA) and ethoxydi(ethylene glycol) methacrylate (DEGEMA) (Scheme 2) allowed for control of the LCST of the polymer through feed ratios, while pH sensitivity was imparted through the incorporation of $\sim 10\text{ mol } \%$ *N,N*-diethylaminoethyl methacrylate (DEAEMA).

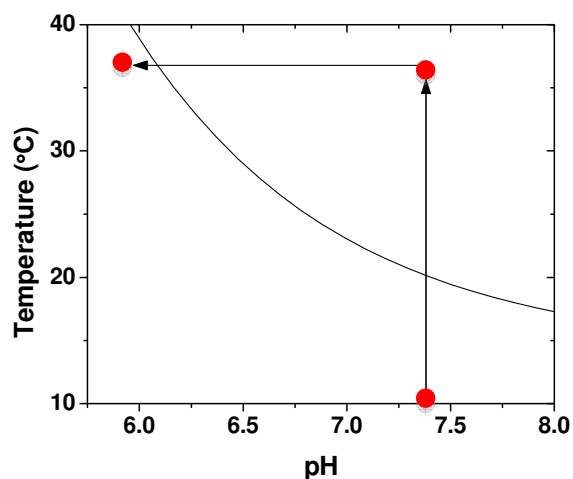
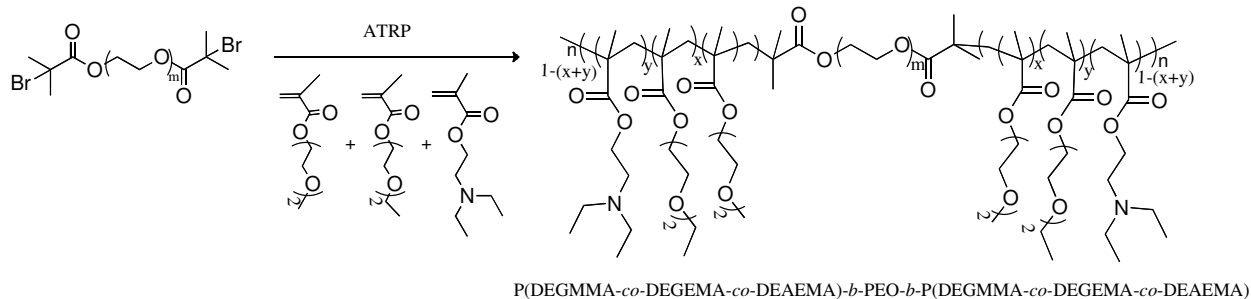


Figure 1. Projected path of aqueous polymer solution as drug molecule matrix. A solution of a thermo- and pH-sensitive ABA triblock copolymer loaded with drug molecules and adjusted to the physiological pH (7.4) and a temperature of $10\text{ }^{\circ}\text{C}$ is injected into the body. The solution becomes a gel as it assumes the physiological temperature of $37\text{ }^{\circ}\text{C}$. As the gel comes in contact with diseased tissues, lower pH values raise $T_{\text{sol-gel}}$, resulting in increased gel erosion in these areas, and, therefore, a more rapid delivery of drug molecules to the diseased tissues.

Scheme 2. Synthesis of a Thermo- and pH-Sensitive ABA Triblock Copolymer



Experimental Section

Materials. Methoxydi(ethylene glycol) methacrylate (DEGMA, or di(ethylene glycol) monomethyl ether methacrylate) and ethyl 2-bromoisobutyrate (EBiB, Aldrich) were dried over calcium hydride and distilled under reduced pressure. CuBr (98 %, Aldrich) was purified according to the procedure described in the literature and was stored in a desiccator.^{34,35} 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97 %, Aldrich), dichloromethane (DCM), and anisole (99 %, Acros) were used without further treatment; DCM, when referred to as dry, was processed via the Grubbs system. *N,N*-Diethylaminoethyl methacrylate (DEAEMA) (99 %, Aldrich) was passed through a basic aluminum oxide column prior to use. Poly(ethylene glycol) (HO-PEO-OH) with a molecular weight of 20,000 g/mol was obtained from Aldrich. All other chemicals were purchased from either Aldrich or Fisher and used without further purification.

General Characterization. Size exclusion chromatography (SEC) was carried out at ambient temperature using PL-GPC 50 Plus (an integrated GPC/SEC system from Polymer Laboratories, Inc) with a differential refractive index detector, one PSS GRAL guard column (50 x 8 mm, 10 μ m particles, Polymer Standards Service-USA, Inc.), and two PSS GRAL linear columns (each 300 x 8 mm, 10 mm, molecular weight range from 500 to 1,000,000 according to Polymer Standards Service-USA, Inc.). The data were processed using CirrusTM GPC/SEC software

(Polymer Laboratories, Inc.). *N,N*-Dimethylformamide (DMF) was used as the carrier solvent at a flow rate of 1.0 mL/min. Standard monodisperse polystyrenes (Polymer Laboratories, Inc.) were used for calibration. ^1H NMR (300MHz) spectra were recorded on a Varian Mercury 300 NMR spectrometer and the residual solvent proton signal was used as the internal standard.

Synthesis of Ethoxydi(Ethylene Glycol) Methacrylate (DEGEMA). Di(ethylene glycol) monoethyl ether (51 g, 0.38 mol) was added to a 500 mL three-neck flask and placed under an N_2 atmosphere. The flask was then charged with 30 mL anhydrous dichloromethane (DCM) and triethylamine (29 g, 0.29 mol); the mixture was then allowed to stir. Next, methacryloyl chloride (20.419 g, 0.195 mol) was diluted with ~60 mL anhydrous DCM and added dropwise to the solution, and allowed to stir overnight. The solution was then gravity filtered and washed in 1.0 M solutions of sodium bicarbonate and HCl as well as water with the aid of saturated sodium chloride solution. The product was then dried overnight with anhydrous sodium sulfate. The monomer was then purified both by column chromatography, using ethyl acetate as eluent, and through vacuum distillation, using hydroquinone to prevent thermal polymerization. Finally, the product was washed in 1.0 M sodium hydroxide solution, after which it was dried overnight with anhydrous sodium sulfate. The product was then dried *in vacuo* at room temperature, yielding a colorless liquid. The structure of the monomer was then verified via ^1H NMR spectroscopy. ^1H NMR (CDCl_3) δ (ppm): 6.11 (s, 1H, *cis*-vinyl), 5.55 (s, 1H, *trans*-vinyl), 4.28 (q, 2H, COOCH_2), 3.61 (m, 8H, $\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2$), 1.90 (s, 3H, $\text{CH}_2\text{C}(\text{COO})\text{CH}_3$), 1.24 (t, 3H, CH_2CH_3).

Synthesis of P(DEGMMA-*co*-DEGEMA). Copper(I) bromide (6.0 mg, 0.044 mmol) was placed into a two-necked flask, as were DEGMMA (0.466 g, 2.5 mmol), and DEGEMA (0.757 g, 3.7 mmol), Anisole (1.533 g) was then added, followed by 1,1,4,7,10,10-hexamethyltriethylenetetramine (0.017 g, 0.072 mmol). Ethyl 2-bromoisobutyrate (EBIB) (4.0 mg, 0.019 mmol) was injected into the reaction mixture immediately before the solution was

frozen by means of a liquid N₂ bath. Degassing was then performed via three freeze-pump-thaw cycles, with several purges of N₂ gas employed in each cycle. The flask was then placed in a 75 °C oil bath. The polymerization was monitored by ¹H NMR spectroscopy and carried out for 119 min, reaching a monomer conversion of 38.7 %, calculated from the ¹H NMR spectrum using peak at 5.58 ppm in reference to the peaks between 4.1 and 4.4 ppm. The reaction was then halted, first by dilution with tetrahydrofuran (THF) after removal from heat and then by the removal of the copper catalyst with filtration through a basic alumina column. The polymer was then precipitated several times in 9:1 (v:v) hexanes and ethyl ether and characterized by SEC and ¹H NMR.

Preparation of an Aqueous Solution of P(DEGMMA-co-DEGEMA) and Cloud Point Measurement. A P(DEGMMA-*co*-DEGEMA) solution in DCM was added to a preweighed vial and concentrated via rotavapor. The vial was then placed into a round bottom flask, which was then immersed in a 50 °C oil bath. The polymer was then dried under vacuum for 197 min. The dry polymer was then weighed, dissolved in water, transferred to a larger vial and diluted to form a 0.5 wt % solution; quantitative transfer was insured by filling the initial container with the water to be added and sonicating it at 0 °C using a Fisher Scientific Model B200 Ultrasonic Cleaner before addition. Cloud point was then determined by visual inspection using a Fisher Scientific isotemp.

Synthesis of a Random Copolymer of DEGMMA, DEGEMA, and DEAEMA. Copper(I) bromide (0.015 g, 0.10 mmol), DEGMMA (0.866 g, 4.6 mmol), DEGEMA (1.420 g, 7.0 mmol), and DEAEMA (0.248 g, 1.3 mmol) were placed into a two-necked flask. Anisole (2.718 g) was then added, followed by 1,1,4,7,10,10-hexamethyltriethylenetetramine (0.036 g, 0.16 mmol). Ethyl 2-bromoisobutyrate (EBIB) (0.015 g, 0.077 mmol) was injected into the reaction mixture immediately before the solution was frozen by means of a liquid N₂ bath. Degassing was then

performed via three freeze-pump-thaw cycles, with several purges of N₂ gas employed in each cycle. The flask was then placed in a 75 °C oil bath. The polymerization was monitored by ¹H NMR spectroscopy and carried out for 98 min, reaching a monomer conversion of 63.7 %. The reaction was then halted, first by dilution with THF after removal from heat and then by the removal of the copper catalyst with filtration through a basic alumina column. The polymer was then precipitated several times in 9:1 (v:v) hexanes and ethyl ether and characterized by SEC and ¹H NMR.

Preparation of Aqueous Solutions of P(DEGMMA-co-DEGEMA-co-DEAEMA) and pH Dependence of Cloud Point. A P(DEGMMA-co-DEGEMA-co-DEAEMA) solution in DCM was added to a preweighed vial and concentrated via rotavapor. The polymer was then dried in a manner similar to that of P(DEGMMA-co-DEGEMA) above. The dry polymer was then weighed and dissolved in 11 mM KH₂PO₄. A concentration of 0.5 wt % was obtained in a fashion similar to that of the P(DEGMMA-co-DEGEMA) solution described above. Solutions at varying pH values were obtained by adjusting this stock solution to the desired pH, using small amounts of 1.0 M KOH and HCl solutions, and collecting a sample before pH was adjusted again. pH values were measured with an Accumet AB15 pH meter from Fisher Scientific, calibrated with pH = 4.01, 7.00, and 10.01 standard buffer solutions at 0 °C; the polymer solution was allowed to equilibrate at least 15 min before pH values were recorded. The cloud point of each pH-adjusted solution was then measured by visual inspection using a Fisher Scientific isotemp.

Synthesis of Difunctional PEO Macroinitiator (Br-PEO-Br) Poly(ethylene oxide) (HO-PEO-OH) (21.011 g, 1.051 mmol) with a molecular weight of 20,000 g/mol was placed into a 500 mL three-necked flask and dissolved in 250 mL toluene. The trace amount of water present in the HO-PEO-OH was removed azeotropically, at atmospheric pressure, through the distillation

of approximately 150 mL toluene. Upon cooling, triethylamine (1.324 g, 13.1 mmol) was added, and the resultant solution was stirred under a nitrogen atmosphere for 30 min. Afterwards, 2-bromoisobutyryl bromide (6.1660 g, 26.8 mmol) was added dropwise. The mixture was then stirred overnight, after which the precipitate was removed via vacuum filtration.

The macroinitiator solution was concentrated and precipitated in diethyl ether. The precipitate was then dissolved in dichloromethane and precipitated three times in diethyl ether. pH 8 water, adjusted by means the addition of 1.0 M KOH, was used to dissolve the Br-PEO-Br, which was then extracted into dichloromethane and dried over anhydrous sodium sulfate overnight. The polymer was then precipitated in diethyl ether and recrystallized in ethanol twice. The macroinitiator was then dried under vacuum at 60 °C, resulting in a hard, white solid upon cooling.

Synthesis of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA). Copper(I) bromide (0.011 g, 0.77 mmol) and Br-PEO-Br (0.510 g, 2.6×10^{-5} mol) were placed into a two-necked flask, as were DEGMMA (0.429 g, 2.3 mmol) DEGEMA (0.688 g, 3.4 mmol) and DEAEMA (0.115g, 0.62 mmol). Anisole (5.061 g) was then added, followed by 1,1,4,7,10,10-hexamethyltriethylenetetramine (0.017 g, 0.074 mmol). The solution was then frozen and degassed through suction under vacuum and several purges with N₂ gas. After thawing the solution, the flask was placed in a 60 °C oil bath. The polymerization was monitored by ¹H NMR spectroscopy, and, after 136 min, the polymerization was stopped by opening the flask to air at room temperature and diluting the reaction mixture with THF and passing through a short column of basic aluminum oxide and silica gel, using dichloromethane as eluent. The polymer was then precipitated several times in 9:1 (v:v) hexanes and ethyl ether and characterized by of SEC and ¹H NMR spectroscopy.

Preparation of Aqueous Solution of ABA and Gel Study. A P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) solution in DCM was added to a preweighed vial and concentrated via rotavapor. The vial was then placed into a round bottom flask, which was then immersed in a 50 °C oil bath. The polymer was then dried under vacuum for 190 min before dissolving in water to form a 10 wt % aqueous solution. The pH of this solution was adjusted using 1.0 M HCl and measured with a pH meter. When the desired pH had been obtained, ~90 μ L of the polymer solution was taken for rheological analysis.

Rheology Experiments. Rheological experiments were conducted using a stress-controlled rheometer (TA Instruments Model TA AR2000ex). A cone-plate geometry with a cone diameter of 20 mm, an angle of 2°, and a truncation of 52 μ m was employed; the temperature was controlled by the bottom Peltier plate. In each measurement, ~ 90 μ L of a polymer solution was loaded onto the plate by a micropipette, and the solvent trap filled with water and covered to minimize evaporation. Dynamic viscoelastic properties, specifically the dynamic storage modulus, G' , and dynamic loss modulus, G'' , of polymer solutions were measured by oscillatory shear experiments performed at a fixed frequency of 1 Hz. A temperature ramp was carried out at a rate of 2 °C/min. A strain amplitude of $\gamma = 0.2 \%$ was used in all dynamic tests to ensure that the deformation was within the linear viscoelastic regime.

Results and Discussion

Synthesis of DEGEMA. DEGEMA was successfully synthesized through the reaction of methacryloyl chloride and di(ethylene glycol) monoethyl ether in the presence of triethylamine (Scheme 3). The monomer was purified through column chromatography and vacuum

distillation. The identity of the monomer was confirmed through the ^1H NMR spectroscopy (Figure 2).

Scheme 3. Synthesis of Ethoxydi(ethylene Glycol) Methacrylate (DEGEMA)

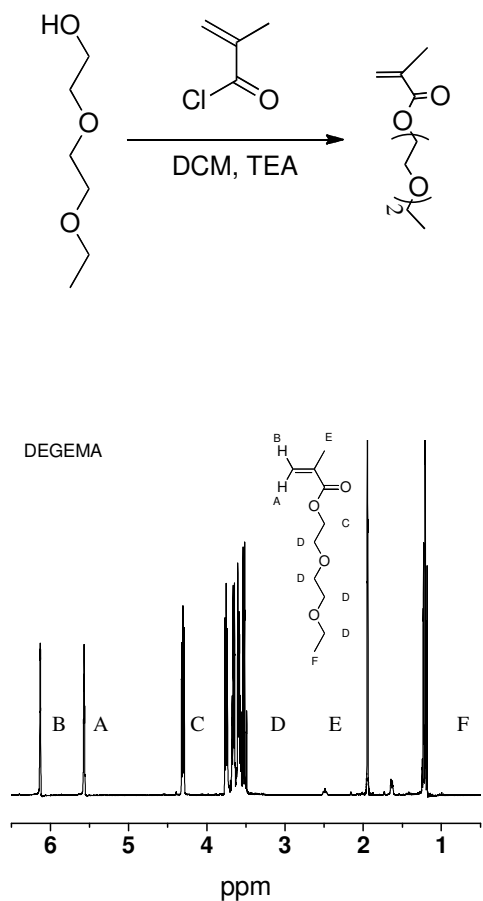


Figure 2. ^1H NMR spectrum of DEGEMA. CDCl_3 was used as solvent in ^1H NMR spectroscopy analysis.

Synthesis of P(DEGMMA-*co*-DEGEMA). P(DEGMMA-*co*-DEGEMA) was synthesized through the ATRP of DEGMMA and DEGEMA (Scheme 4). The obtained P(DEGMMA-*co*-DEGEMA) was found to have an $M_{n, \text{SEC}}$ of 28.3 kDa, and a polydispersity index (PDI) of 1.31 (relative to polystyrene standards). From the monomer conversion of 38.7 %, it was calculated

that the polymer had attained an average degree of polymerization (DP) of 127, composed of 56.8 % DEGEMA units and 43.2 % DEGMMA, close to ratio of 60:40 in the feed. This ratio of repeat units was calculated from the ^1H NMR spectrum (Figure 3) of the purified polymer, specifically from the integrals of the singlet about 3.4 ppm and the triplet at 1.2 ppm, corresponding to the methyl (OCH_3) and ethyl CH_3 (OCH_2CH_3) ether groups on DEGMMA and DEGEMA respectively.

Scheme 4. Synthesis of P(DEGMMA-*co*-DEGEMA)

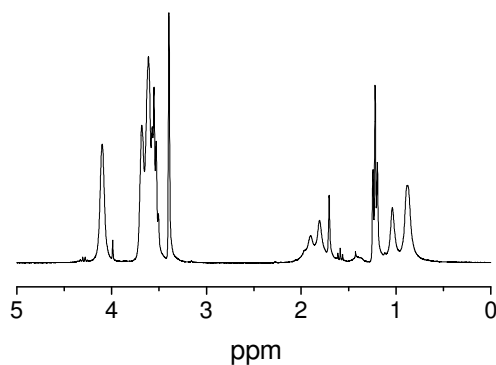
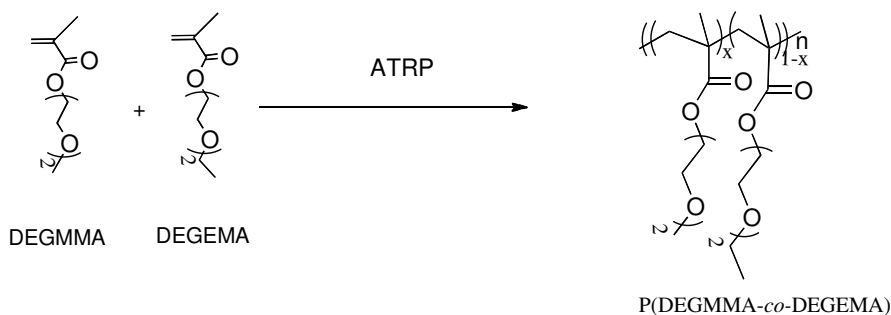


Figure 3. ^1H NMR spectrum of P(DEGMMA-*co*-DEGEMA) using CDCl_3 as solvent.

The selection of the monomer feed ratio employed in this experiment was based upon the known cloud points of PDEGMMA (26 °C) and PDEGEMA (4 °C). It has long been known that random copolymers often exhibit cloud points with respect to the cloud points of the homopolymers of their constituent monomers, a fact shown to largely apply to oligo(ethylene

glycol) methacrylates.³⁸ The cloud point of a 0.5 wt % aqueous solution of this polymer was found to be 11.5 °C, showing a good agreement with the 13.5 °C expected from a totally linear combination. This feed ratio of DEGMMA to DEGEMA was selected for the remainder of the polymerizations in this project, as prior research has shown that ABA triblock copolymers of the manner to be synthesized typically show a $T_{\text{sol-gel}}$ 10 °C higher than the cloud point of random copolymers analogous to their A-blocks, resulting in a gel at room temperature, as desired.

Synthesis of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA). P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) was synthesized by the ATRP of DEGMMA, DEGEMA, and DEAEMA (Scheme 5). Through its SEC trace (Figure 4) the synthesized P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) was found to have an $M_{n,\text{SEC}}$ of 35.1 kDa, and a polydispersity index (PDI) of 1.24 (relative to polystyrene standards). From the monomer conversion of 63.7 %, it was calculated that the polymer had attained an average degree of polymerization (DP) of 107, composed of 51.7 % DEGEMA units, 37.7 % DEGMMA, and 10.6 % DEAEMA, a ratio of DEGEMA to DEGMMA of 58:42. This ratio of repeat units was calculated from the ^1H NMR spectrum (Figure 5) of the purified polymer, specifically from the integrals of the singlet about 3.4 ppm corresponding to $3x$, with x being the fraction of DEGMMA residues as shown in Scheme 5, the triplet at 1.2 ppm to $3y$, and the multiplet ($\text{N}(\text{CH}_2)_3$) spanning 2.48 and 2.71 ppm corresponding to six times the DEAEMA contribution, $1-(x+y)$.

Scheme 5. Synthesis of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)

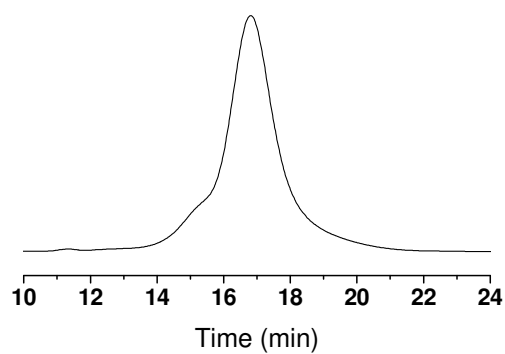
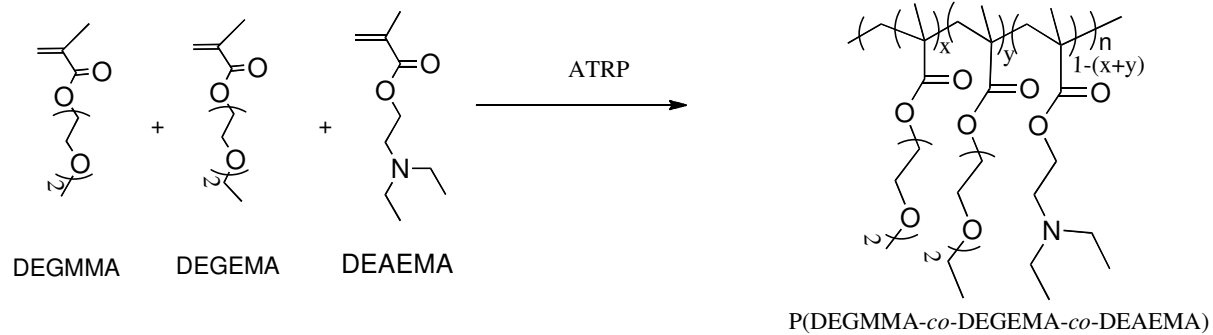


Figure 4. Size exclusion chromatograph of P(DEGMA-co-DEGEMA-co-DEAEMA)

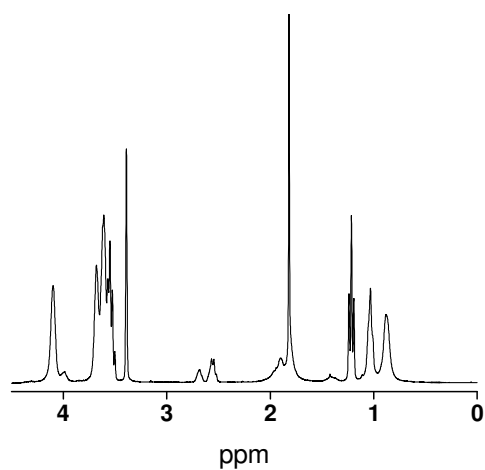


Figure 5. ^1H NMR spectrum of P(DEGMA-co-DEGEMA-co-DEAEMA)

Cloud point measurements revealed the expected dependence upon pH, with cloud point remaining low and roughly constant above a pH of 9 and increasing as pH was lowered, as shown in Figure 6. The dependence of cloud point on pH can be well described as having a functional form of a simple exponential decay, somewhat remarkable observation given the number of factors which contribute to cloud point.

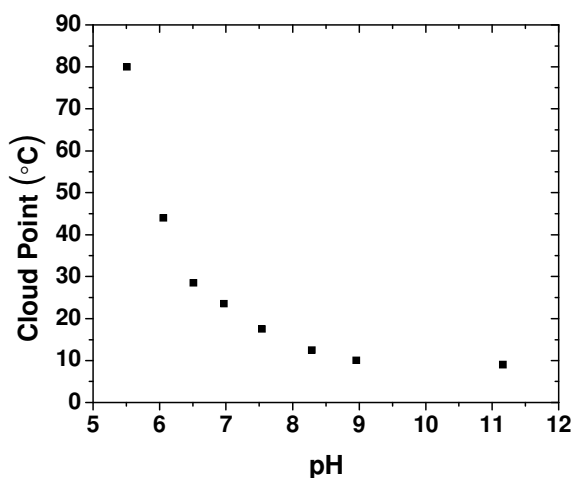


Figure 6. Cloud point of 0.5 wt % P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) at various pH values

Synthesis of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA). A linear, hydrophilic ABA triblock copolymer was synthesized through the ATRP of DEGMMA, DEGEMA, and DEAEMA using a difunctional macroinitiator (Scheme 6). According to its SEC trace (Figure 7) the synthesized P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*co*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) was found to have an $M_{n,SEC}$ of 65.1 kDa, and a polydispersity index (PDI) of 1.22 (relative to polystyrene standards).

The DP and ratio of repeat units were calculated from the ^1H NMR spectrum (Figure 8) of the purified polymer, the later being found, as before, from the integrals of the singlet about 3.4 ppm corresponding to $3x$, the triplet at 1.2 ppm to $3y$, and the multiplet spanning 2.48 and 2.71 ppm corresponding to six times the DEAEMA contribution, $1-(x+y)$. The multiplet spanning from 3.5 to 3.75 ppm was taken to correspond to ethylene glycol hydrogens and, therefore, has an integration of $4m + 6x + 8y$; since n is known to be 455 on average, the average DP of each monomer was able to be calculated, with DEGMMA being 78 repeat units, DEGEMA 93 units, and DEAEMA 22 units.

Scheme 6. Synthesis of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*co*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)

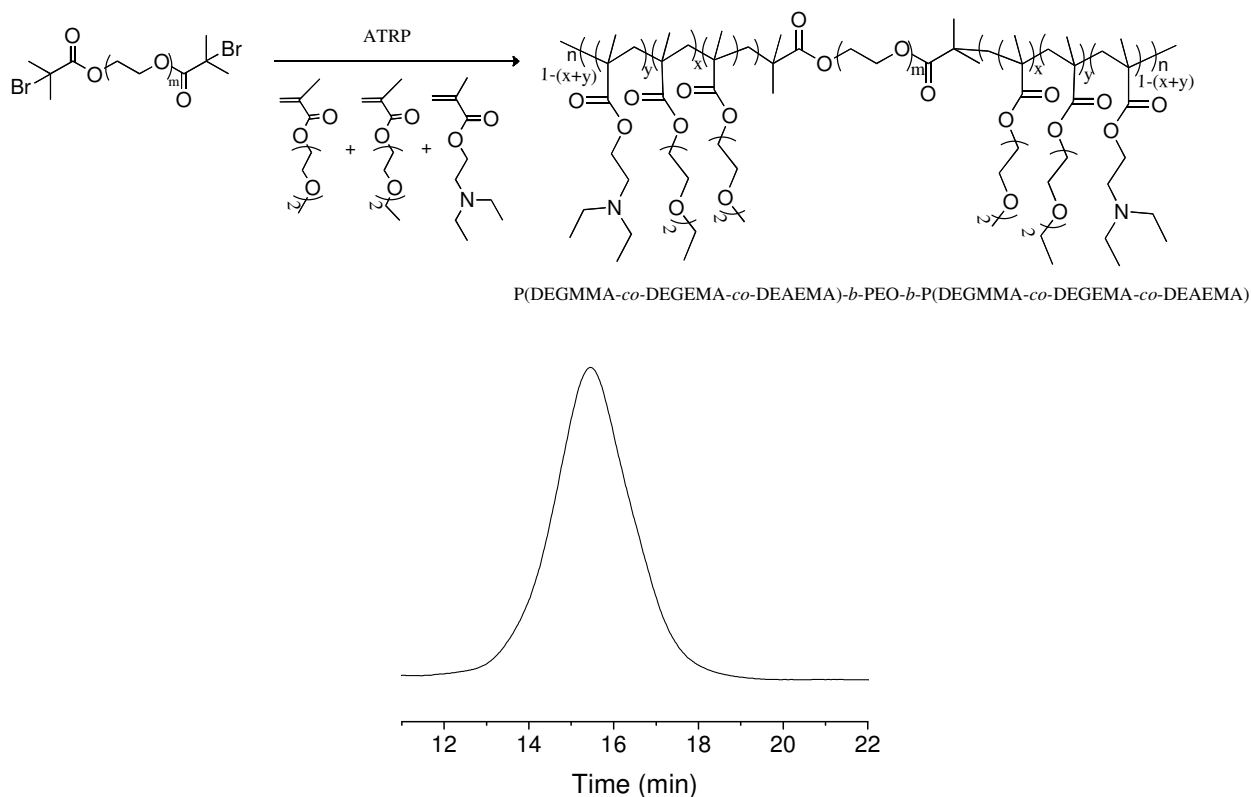


Figure 7. Size exclusion chromatograph of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*co*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)

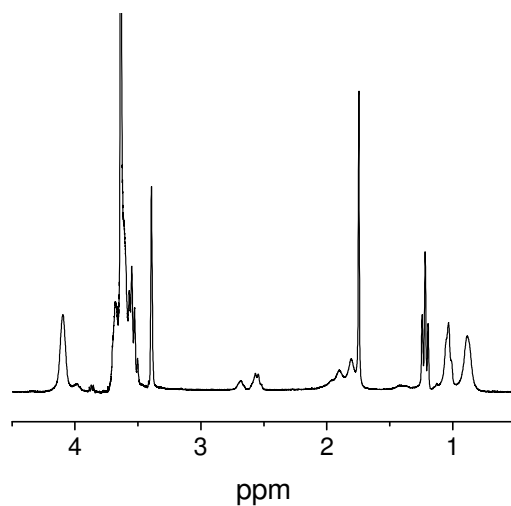


Figure 8. ^1H NMR spectrum of $\text{P}(\text{DEGMMA-}co\text{-DEGEMA-}co\text{-DEAEMA})\text{-}b\text{-PEO-}b\text{-P}(\text{DEGMMA-}co\text{-DEGEMA-}co\text{-DEAEMA})$

In accordance with the low cloud point of the random copolymer of DEGMMA and DEGEMA, a 10 wt % aqueous solution of the ABA triblock copolymer did form a gel at room temperature. Upon visual inspection, the solution was found to be a free-flowing solution at 0 °C and a transparent gel at room temperature, able to support its own weight and that of a stir bar when inverted (Figure 9).

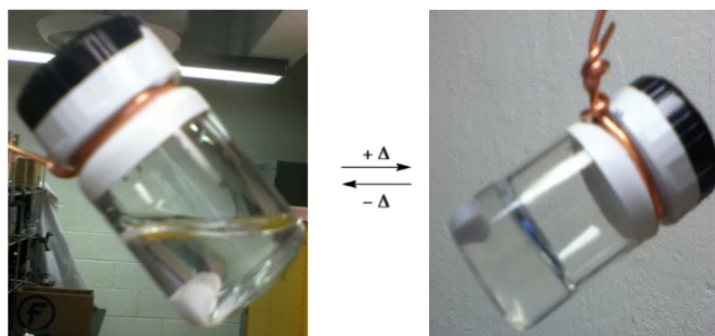


Figure 9. Gelation of 10 wt % $\text{P}(\text{DEGMMA-}co\text{-DEGEMA-}co\text{-DEAEMA})\text{-}co\text{-PEO-}b\text{-P}(\text{DEGMMA-}co\text{-DEGEMA-}co\text{-DEAEMA})$; 0 °C (left) and 25 °C (right)

pH dependence of $T_{\text{sol-gel}}$ was shown by rheological analysis to correspond to the trend observed in the pH-sensitive random copolymer study. The data compiled following each rheometric experiment was used to plot G' , G'' and $\tan(\delta)$, the ratio of G'' to G' , against temperature. The range in which $G' < G''$, the solution can be considered a solution, whereas it is a gel when $G' > G''$, making the temperature at which the two intersect correspond to $T_{\text{sol-gel}}$. $T_{\text{sol-gel}}$ was found to be 22 °C at a pH of 9.65 and 29°C at 8.74, as shown in Figures 10 and 11. While the expected dependence on pH was observed, in that $T_{\text{sol-gel}}$ increased with the decrease in pH, the sharpness of this dependence was surprising; lowering the pH from 8.74 to 8.60 raised $T_{\text{sol-gel}}$ above 100 °C, effectively making the polymer completely water soluble (Figure 12).

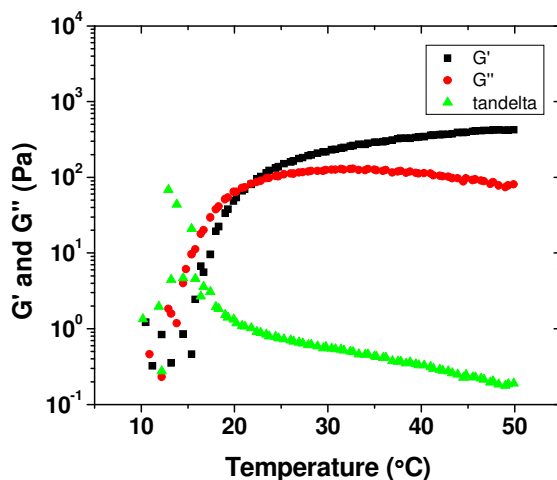


Figure 10. Plot of dynamic storage modulus G' , dynamic loss modulus G'' , and $\tan(\delta)$ versus temperature for the 10.0 wt % aqueous solution of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) at pH = 9.65. The data were collected from a temperature ramp experiment with a heating rate of 2 °C/min. A strain amplitude of 0.2 % and an oscillation frequency of 1 Hz were used.

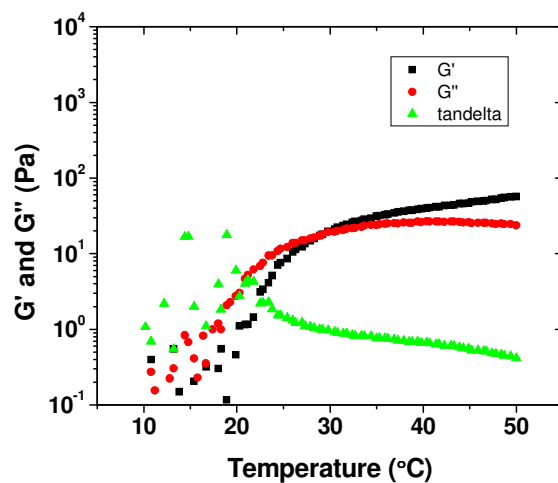


Figure 11. Plot of dynamic storage modulus G' , dynamic loss modulus G'' , and $\tan(\delta)$ versus temperature for the 10.0 wt % aqueous solution of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) at pH = 8.74. The data were collected from a temperature ramp experiment with a heating rate of 2 °C/min. A strain amplitude of 0.2 % and an oscillation frequency of 1 Hz were used.

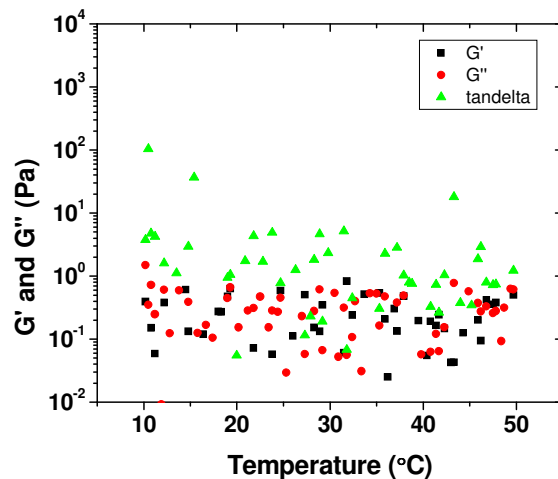


Figure 12. Plot of dynamic storage modulus G' , dynamic loss modulus G'' , and $\tan(\delta)$ versus temperature for the 10.0 wt % aqueous solution of P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA)-*b*-PEO-*b*-P(DEGMMA-*co*-DEGEMA-*co*-DEAEMA) at pH = 8.60. The data were collected from a temperature ramp experiment with a heating rate of 2 °C/min. A strain amplitude of 0.2 % and an oscillation frequency of 1 Hz were used.

Conclusions

Overall, the design of a thermo- and pH-responsive gelation system consisting of tertiary amine containing ABA triblock copolymers was successful. A 10 wt % ABA solution in water formed a gel at room temperature, and pH sensitivity was observed both in random copolymer studies and in ABA rheological analysis. The sharpness of the $T_{\text{sol-gel}}$'s dependence on pH was unexpected; the extreme transition at pH 8.6 also precludes this system's use in most biological applications. Still, the more subtle effect observed at higher pH values suggests that employing a tertiary amine-containing monomer with a higher pK_b , such as *N,N*-diisopropylaminoethyl methacrylate or *N,N*-di-*tert*-butylaminoethyl methacrylate, could be employed in future work to remedy this issue.

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